Niger. J. Physiol. Sci. 36 (June 2021): 67 – 76
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full Length Research Article

Kolaviron Protects Rats from Cognitive Decline Induced by Lipopolysaccharide in Wistar Rat

***Onasanwo S.A.[1], Adebimpe-John O.E.[1], Olopade F.E.[2] and Olajide O.A.[3]**

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria
[2]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria
[3]Department of Pharmacy, School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, United Kingdom

Summary: Kolaviron **(**Kol-v) is a mixture of bioflavonoid from the seed of *Garcinia kola*, and has been previously shown to inhibit neuro-inflammation in Lipopolysaccharide (LPS)-activated BV-2 microglia. In this study, we investigated neuroprotective effects of Kol-v in LPS-induced memory impairment in Wistar rat. Wistar rats (225-250) g were used in this study. Memory impairment was induced with the systematic administration of 250µg/mg LPS. The effect of Kol-v on cognition and learning processes were assessed using the behavioral responses in the Morris water maze model; Effects of LPS on bodily activities were assessed by biochemical assays before and after treatment. Intra-peritoneal administration of LPS reduced the core body temperature, cognitive and locomotor process. Kol-v ameliorated the effect LPS on the core body temperature by restoring it back to normal, and significantly improved the cognitive and learning processes. Kol-v significantly increased the level of SOD and CAT and reduction in the levels of NO, GSH, and MDA. Kolaviron showed significant anti-inflammatory potentials through its protection against cognitive decline and oxidative properties induced by lipopolysaccharide in the laboratory Wistar rat.

Keywords: *Cognition, Kolaviron, Lipopolysaccharide, Memory Impairment, Neuro-inflammation, Oxidative stress*

**Address for correspondence: sa.onasanwo@mail.ui.edu.ng; Tel: + 234 8060711936, Fax: + 02-8103043*

Manuscript received- September 2020; Accepted- March, 2021

INTRODUCTION

Cognition involves processes in the brain that includes the ability to learn, remember, and make judgments. When cognition is impaired, it generates great impact on an individual's overall health and well-being. Cognitive decline ranges from mild cognitive impairment to dementia, a form of diminution in capabilities severe enough to meddle with daily life activity. Several pathophysiological mechanisms have been suggested to contribute to neuronal damage in many neurodegenerative disorders. One of the most investigated mechanisms in these conditions is glia-dependent neuro-inflammatory mechanisms, which also include astrocytes, the complement system, as well as cytokines and chemokines (Van *et al*., 2016). Cognitive impairment leads to trouble in remembering, learning new things, concentrating, or making decisions that affect everyday life which leads to reduction in quality of life and is related to neurodegenerative disorders. However, there is a correlation between these diseases and oxidative stress. Recent studies have also established the genetic risk factors in the role of inflammation in memory and learning deficits, since disorders like Alzheimer's disease are connected with increased levels of pro-inflammatory cytokines combined with decreased levels of anti-inflammatory cytokines (Barrientos *et al.*, 2009).

Oxidative stress is a pathophysiological mechanism that is closely associated with neurodegenerative disorders. It can be defined as an imbalance between reactive oxygen (ROS) and nitrogen species (RNS) and attenuated antioxidant defenses. The increased oxidative activities such as generation of superoxide anion ($O2^-$) and hydrogen peroxide (H_2O_2), and subsequently reactive oxygen species (ROS) can result in neuronal oxidative damage (Sorce *et al.*, 2017).

Kol-v is a mixture of three compounds - *Garcinia biflavonoid GB1, GB2 and Kola flavanone* (Iwu *et al*, 1990). Bi-flavonoids are the most abundant compounds in *Garcinia kola* a popular West African edible seed, while the kola flavones are the major components of Kol-v. *Garcinia biflavonoids* have been attributed to their ability to scavenge free radicals, induce detoxification, inhibit stress response proteins and interfere with DNA binding activities of some transcription factors. A number of studies have reported that Kol-v exhibited antioxidant anti-inflammatory activity *in vivo* and *in vitro* (Abarikwu 2014; Farombi *et al.*; 2009 Olaleye *et al*., 2010).

Indications about the ability of Kol-v to protect neurons from damage were reported in studies involving neurotoxicity induced with environmental chemicals including atrazine (Abarikwu *et al.*, 2011), vanadium (Igado *et al.*, 2012) and sodium azide (Olajide *et al.*, 2015). It has been reported earlier that Kol-v inhibits neuroinflammation and microglia-mediated neurotoxicity through mechanisms involving Nrf2/ARE antioxidant pathway (Onasanwo *et al.*, 2016). The anti-inflammatory and anti-oxidative potentials of Kol-v are yet to be properly explored in laboratory rodents induced with lipopolysaccharide. This study was

designed to investigate the impact of Kol-v on lipopolysaccharide-induced cognitive decline and oxidative stress in Wistar rat.

Plate 1:
Garcinia kola in its Pod (bitter kola, a family of *Guttiferae*) from Ife (Adebimpe-John E.O Original 2014)

MATERIALS AND METHODS

Extraction of Kolaviron: Kolaviron was extracted from the seeds of *Garcinia kola* using a widely reported protocol (Iwu 1985) and earlier described (Onasanwo *et al.*, 2016). Briefly, *Garcinia kola* nuts were purchased from local market in Ile-Ife, Nigeria. The seeds were peeled and air-dried in the laboratory. 8.2kg air dried seeds were ground into powdered form. The powdered seeds were extracted with n-hexane, in a soxhlet extractor. The de- fatted; dried mass was repacked and then extracted with methanol in a soxhlet extractor. The extract was concentrated and was diluted to twice its volume in distilled water and partitioned with chloroform. The concentrated chloroform fraction gave a yellow-brown solid known as Kolaviron, which was allowed to dry in oven (40˚C), and ground to powdered form. Kolaviron was suspended in corn oil for *in vivo* experiments. The rationale for the doses of Kolaviron used was in relation to the least intake of 3 seeds of the raw consumption of Garcinia kola daily. In comparison, all doses of the extracts used in this study were expressed in terms of the dried sample.

Animals: Wistar rats (225–250g) were used in these experiments, and were acclimatized for two weeks. After acclimatization of two weeks the animals were randomly divided into six study groups of (6) rats per group. The animals were fed with standard rat pellet, and were given water liberally. Animals were housed in clean plastic cages under natural light and dark cycle, and at room temperature. The negative control group received corn-oil only, positive control group were induced with LPS and received nothing, while other groups were treated with varying concentration of Kolaviron (50mg/kg, 100mg/kg, 200mg/kg) and Sulindac sulfide 100ug/kg respectively. Animals were taken care of according to the rules and guidelines of the National Institute of Health (NIH) for laboratory animal care and use. The proposal and use was approved by the University of Ibadan Animal Ethics Review Committee (Number UI-ACUREC/17/0024).

Body temperature: Four days prior to the experiment, the rat's temperature was taken at days 1, 3, 5, 7 and four hours after injection of either LPS or saline and on days 9 and 11 before sacrificing them. Body temperature was recorded using a rectal digital thermometer probe (WPI, Sarasota FL) at the same time each day (8:00 -9:00 hours) to minimize variation due to circadian rhythm

Behavioral test (Morris Water Maze Model): The Morris water maze test is used to assess spatial memory and non-spatial discrimination learning, and was conducted as described by (Morris, 1984). Rats were administered 50mg/kg, 100mg/kg and 200mg/kg Kol-v followed by LPS (250 µg/kg; i.p.) for 7 days. Animals in the vehicle control group received corn oil, while animals in the LPS control group did not receive any treatment. On the 4th day of treatment, learning and memory were evaluated using the Morris water maze test. The water maze consisted of a circular pool of water (150 cm in diameter) with a circular platform of 10 cm diameter and 1 cm below the water surface. The animals had two acquisition trials per day for 3 days during which they were placed in water and expected to find the hidden escape platform. This was taken as a measure of learning ability of the rats. On the last day, the platform was removed and the length of time the rats spent in the quadrant and distance travelled before getting to the region of the platform was recorded.

Preparation of Brain Tissues for Biochemical Assays: The animals were sacrificed firmly through cervical dislocation and the brains were immediately removed while the animal was still alive, washed in potassium chloride buffer and left at 4°C for 30 min. Thereafter, the whole brain was weighed and blotted with 10 % w/v phosphate buffer (0.1 M, pH 7.4), followed by homogenization to obtain the post mitochondrial fraction. Protein concentrations of various samples were determined using the Lowry method (Lowry *et al*., 1951) using bovine serum albumin as a standard. A breakdown product of lipid peroxidation thiobarbituric acid reactive substances (TBARS) was assayed as malondialdehyde (MDA) and measured according to the method of (Beuge *et al*., 1978). The concentration of the reduced glutathione (GSH) in the brain post mitochondrial supernatant was estimated using the method of (Jollow *et al*.,1974) and the levels of total SOD activity in the tissues were determined by the method of (Misra *et al*.,1972). Nitric oxide level was determined using the Griess method (Griess *et al*.,1879) while the catalase activity was determined by the method of Clairborne (Clairborne., 1995).

Statistical Analysis: Data are presented as mean ± S.E.M. Statistical analysis was done using one-way analysis of variance (ANOVA) test, followed by Student Newman-Keuls test for multiple comparisons. The level of significant difference between the groups was evaluated at $p< 0.05$. All statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad Software, Inc., San Diego, CA).

RESULTS

Effects of Kolaviron on lipopolysaccharide-induced changes in body weight: The weight changes of animals

were monitored during the seven days of pre-treatment and post-treatment with fourteen days of pre-post treatment. At the beginning of the treatment, a significant weight loss that is reflected by the LPS injection was observed in all the groups (Figure 1a, b and c) however, the animals seemed to have recovered from the LPS treatment and showed weight gain with the vehicle treated animals. Kolaviron at the higher dose (200mg/kg) showed an increased weight with the vehicle animals, supplementation with Kolaviron restored the relative body weight.

Effects of Kolaviron on lipopolysaccharide-induced changes in the body temperature: There was a marked drop in temperature at day 1and at day 7, four hours after LPS injection (Fig 1d,e and f), it showed that the LPS treatment induced a significant reduction of body temperature after the first dose of LPS On subsequent treatment, there was a significant increase in the body temperature after few days of LPS induction p value < 0.0045(A), the animals showed a difference in their core body temperature compared to the vehicle group. However, the result was significantly different at p < 0.0001.

Effects of Kolaviron on learning and memory tasks in rats: The results of the acquisition training (Figure2a, 3a, 4a and 2b, 3b, 4b; invisible platform trial) of six trials (two times per day) for 3 days to measure the escape latency and path-length showed a gradual reduction in percentage time along the days between the groups during the training trial.

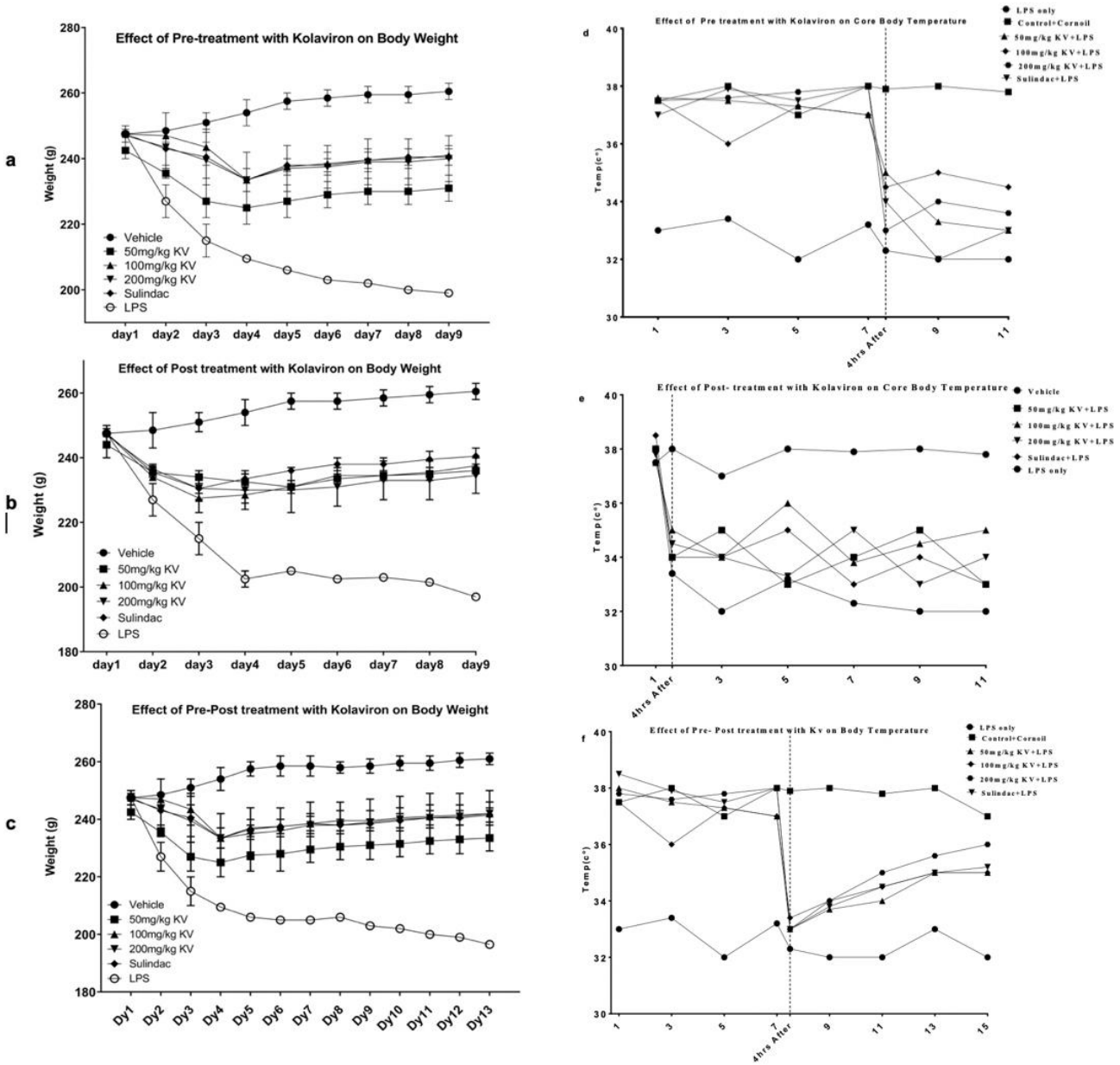

Figure 1:
Effects of Kol-v on single dose of LPS (250µg/kg on the body weight and core body temperature (a)Pre-treatment (b) Post-treatment and (c) Pre-Post-treatment. Following treatment with Kol-v (50mg/kg, 100mg/kg and 200mg/kg) for 7days and 14 days. Each value is mean ± S.EM. *Significantly different from vehicle (negative control group). Significance was verified with one-way ANOVA test, followed by Student Newman-Keuls (SNK) test for multiple comparisons. P < 0.001; N = 6

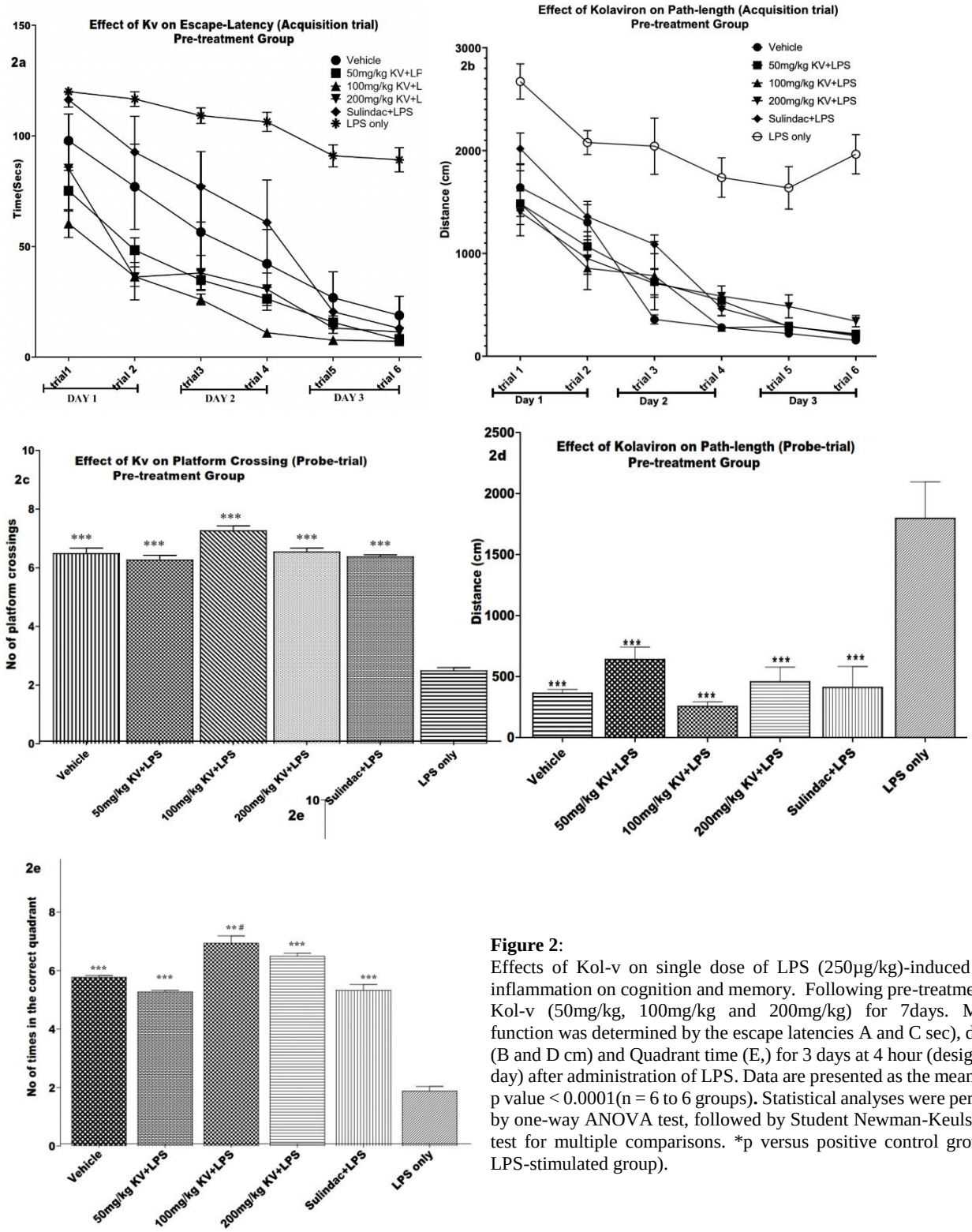

Figure 2:
Effects of Kol-v on single dose of LPS (250µg/kg)-induced neuro-inflammation on cognition and memory. Following pre-treatment with Kol-v (50mg/kg, 100mg/kg and 200mg/kg) for 7days. Memory function was determined by the escape latencies A and C sec), distance (B and D cm) and Quadrant time (E,) for 3 days at 4 hour (designated 1 day) after administration of LPS. Data are presented as the mean ± S.D. p value < 0.0001(n = 6 to 6 groups)**.** Statistical analyses were performed by one-way ANOVA test, followed by Student Newman-Keuls (SNK) test for multiple comparisons. *p versus positive control group (the LPS-stimulated group).

The results were averaged across two trials per day for 3days. The results of the probe trial (Figure 2c, 3c, 4c; non-visible platform trial) LPS treated group (positive control) showed an increase in time to locate the platform when compared to other groups that has been pre-treated with Sulindac sulfide (100µg/kg,) and Kol-v (50mg/kg, 100mg/kg and 200mg/kg) they took a shorter time to locate the platform along the experimental days. The LPS-treated rat travelled a longer distance to reach the platform while the other groups travelled a relative short distance to reach the platform (Figure2d, 3d, 4d).The number of time they entered into the quadrant (quadrant time) was reduced in the LPS group compared to other groups with higher frequency of entering the quadrant. The effect Kol-v was seen to be effective at 100mg/kg for Pre-treatment, 50mg/kg for Post-treatment and 200mg/kg for Pre-Post treatment (Figure 2e, 3e, 4e).It is considered that these differences between the groups reflected the differences in timing of cognitive decline.

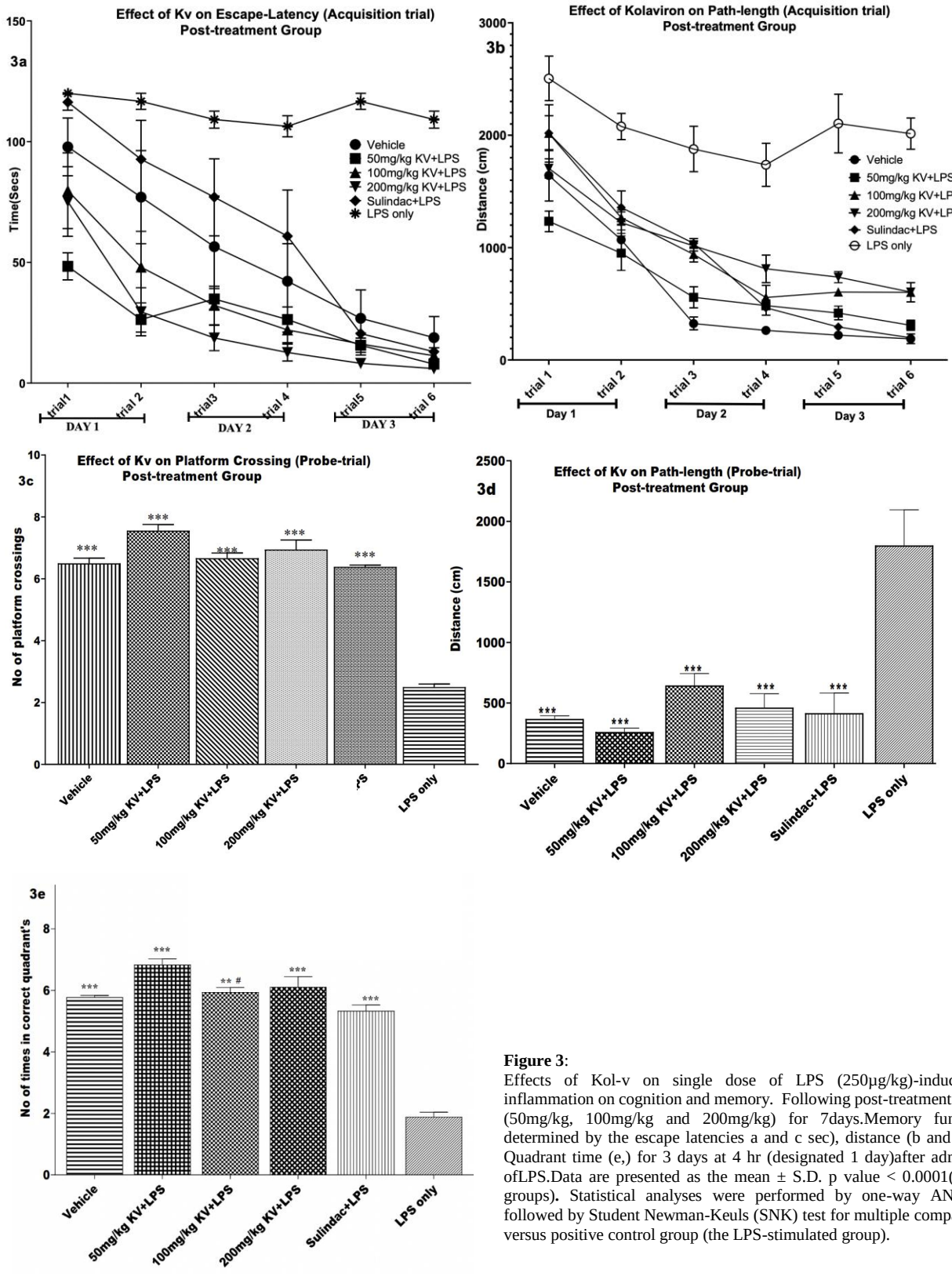

Figure 3:
Effects of Kol-v on single dose of LPS (250µg/kg)-induced neuro-inflammation on cognition and memory. Following post-treatment with Kol-v (50mg/kg, 100mg/kg and 200mg/kg) for 7days.Memory function was determined by the escape latencies a and c sec), distance (b and d cm) and Quadrant time (e,) for 3 days at 4 hr (designated 1 day)after administration ofLPS.Data are presented as the mean ± S.D. p value < 0.0001(n = 6 to 6 groups). Statistical analyses were performed by one-way ANOVA test, followed by Student Newman-Keuls (SNK) test for multiple comparisons. * p versus positive control group (the LPS-stimulated group).

Effects of Kolaviron on the MDA Level in cognitive decline induced by LPS: The effect of pre-treatment, post-treatment and pre-post treatment with varying concentration of Kol-v (50mg/kg, 100mg/kg, 200mg/kg) and Sulindac sulfide (100µg/kg, orally) on the MDA level was examined as shown in (Figure 5a, b and c).

Effects of Kolaviron on the GSH Level in cognitive decline induced by LPS: We examined the effect of pre-post treatment with varying concentration of Kol-v (50mg/kg, 100mg/kg, 200mg/kg) and Sulindac sulfide (100µg/kg, orally) on the GSH level. As shown in (Figure 6a, b and c).

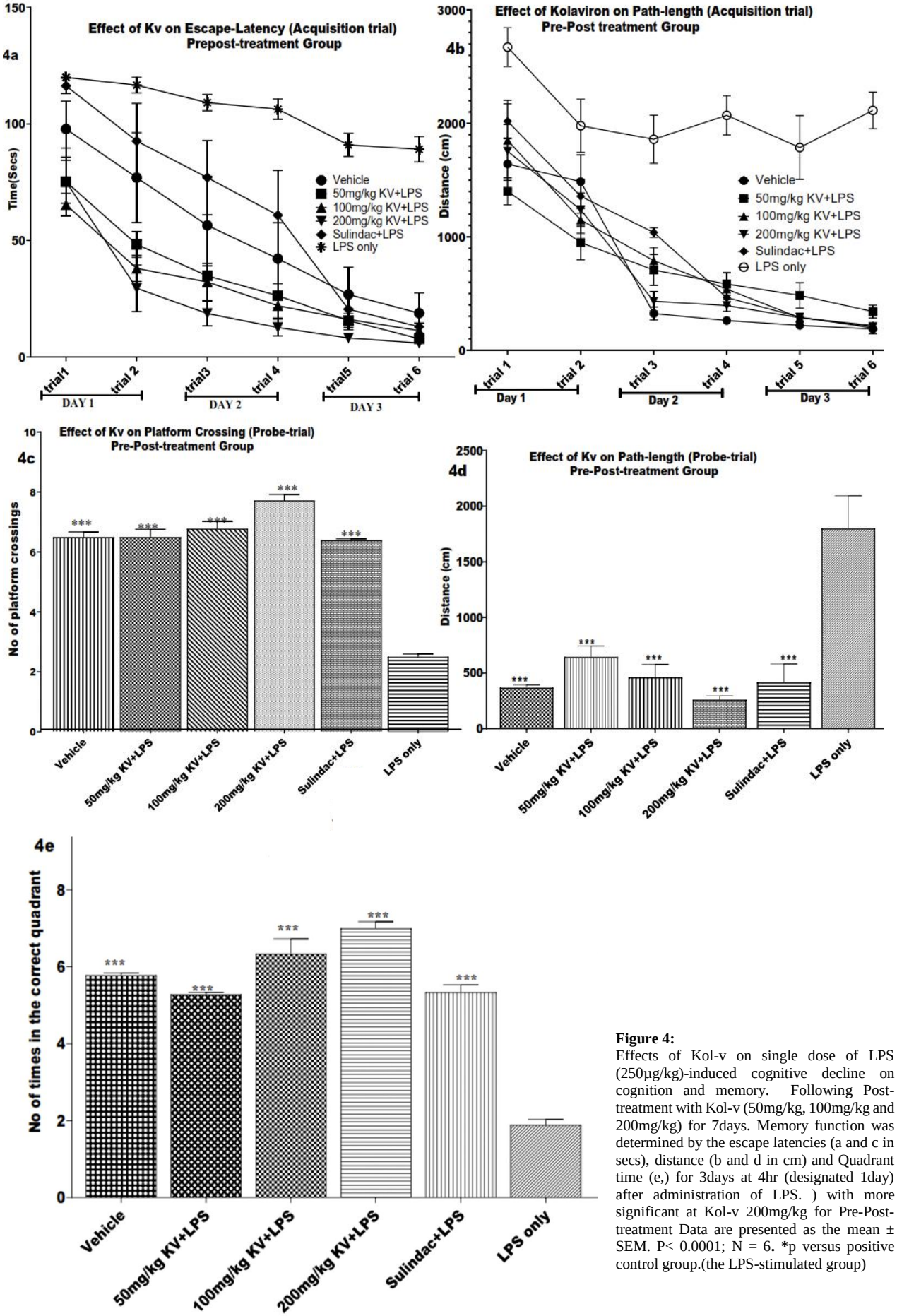

Figure 4:
Effects of Kol-v on single dose of LPS (250µg/kg)-induced cognitive decline on cognition and memory. Following Post-treatment with Kol-v (50mg/kg, 100mg/kg and 200mg/kg) for 7days. Memory function was determined by the escape latencies (a and c in secs), distance (b and d in cm) and Quadrant time (e,) for 3days at 4hr (designated 1day) after administration of LPS.) with more significant at Kol-v 200mg/kg for Pre-Post-treatment Data are presented as the mean ± SEM. P< 0.0001; N = 6**.** *p versus positive control group.(the LPS-stimulated group)

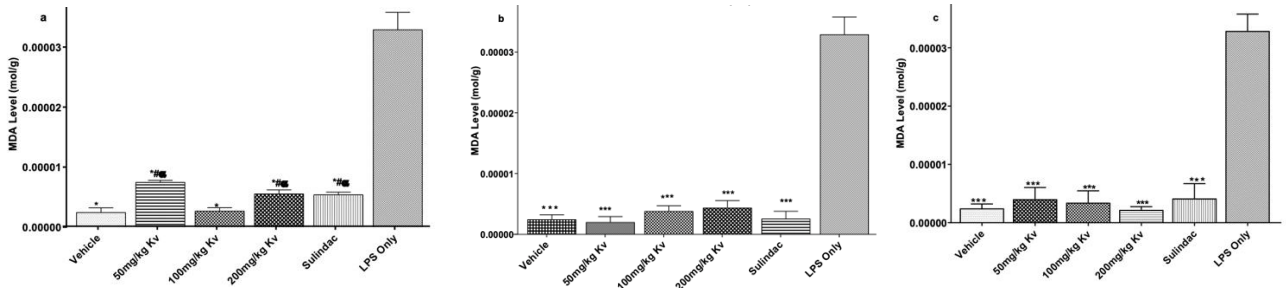

Figure 5:
Effects of Kol-v on single dose of LPS (250µg/kg)-induced cognitive decline on MDA analysis .This result shows the lipid peroxide levels were high in the case of negative control animals (LPS group) which were significantly lowered during the administration of varying concentration of Kolaviron especially at 100mg/kg for Pre-treatment, 50mg/kg for Post-treatment and 200mg/kg for Pre-Post treatment. *****p versus positive control group (the LPS-stimulated group) #p versus negative group (the Vehicle Corn oil only), **ᵅ**p versus 100mg/kg Kol-v. Values are mean ± SEM P< 0.0001; N = 6.

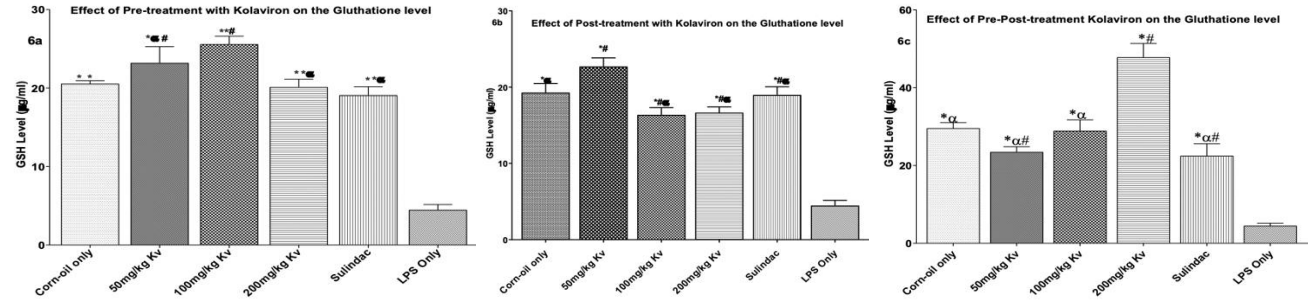

Figure 6:
Effects of Kol-v on single dose of LPS (250µg/kg)-induced cognitive decline on GSH level. This results shows that cognitive decline was stimulated by a significant reduction in the level of the reduced glutathione in the case of negative control animals (LPS group) which were significantly increased by the administration of pre-treatment with Kol-v especially at 100mg/kg for Pre-treatment,50mg/kg for Post-treatment and 200mg/kg for Pre-Post treatment. *****p versus positive control group (the LPS-stimulated group) #p versus negative group (the Vehicle Corn oil only), **ᵅ**p versus 50mg/kg,100mg/kg and 200mg/kg Kol-v respectively. Values are mean ± SEM P< 0.0001; N = 6.

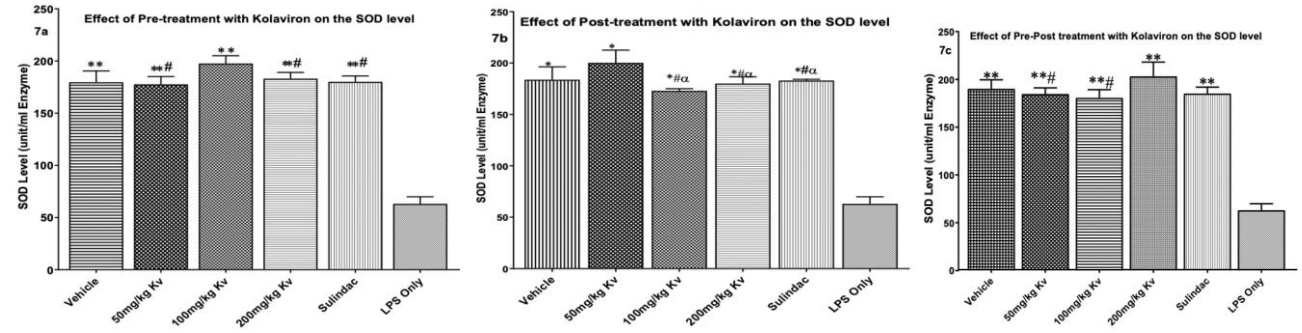

Figure 7:
Effects of Kol-v on single dose of LPS (250µg/kg)-induced cognitive decline on SOD analysis This result shows the SOD level was low in the case of negative control animals (LPS group) which were significantly increased after post-treatment with Kol-v especially at 100mg/kg for Pre-treatment, 50mg/kg for Post-treatment and 200mg/kg for Pre-Post treatment*****p versus positive control group (the LPS-stimulated group) #p versus negative group (the Vehicle Corn oil only), **ᵅ**p versus 100mg/kg Kol-v. Values are mean ± SEM P < 0.0001; N = 6.

Effects of Kolaviron on the Nitric-oxide (NO) Level in cognitive decline induced by LPS*:* The interest in measuring Nitric-Oxide level in biological tissues and fluids remains strong as it is an inflammatory marker. As shown in (Figure 8a, b and c).

Effects of Kolaviron on the Catalase (CAT) Level in cognitive decline induced by LPS*:* The catalase activity on the effect of treatment with varying concentration of Kolaviron (50mg/kg, 100mg/kg, 200mg/kg) and Sulindac sulfide (100µg/kg, orally) on cognitive decline induced by LPS was examined. As shown in (Figure 9a, b and c).

DISCUSSION

Intra-peritoneal lipopolysaccharide (LPS) treatment has been connected with cognitive deficits in rodents, and this has be observed in learning and memory tasks in the Morris water maze (Arai*et al*., 2001; Rosi *et al*. 2006S).

In this study, rats were administered with LPS (250 µg/kg) and were subsequently tested in the Morris water maze, the memory enhances effect and anti-inflammatory properties of Kol-v, a defatted seed extract of *Garcinia kola*, was investigated. We have shown here that Kolaviron exhibited a high level of anti-oxidative properties and very strong anti-inflammatory activities when compared to a standard reference drug, Sulindac sulfide. Kol-v also been interlaced in the neuroprotective role against gamma-radiation-induced brain injury (Adaramoye, 2010), 3-nitro- propionic and methamphetamine-induced neurotoxicity (Nwoha *et al*., 2007; Ijomone *et al*., 2012).

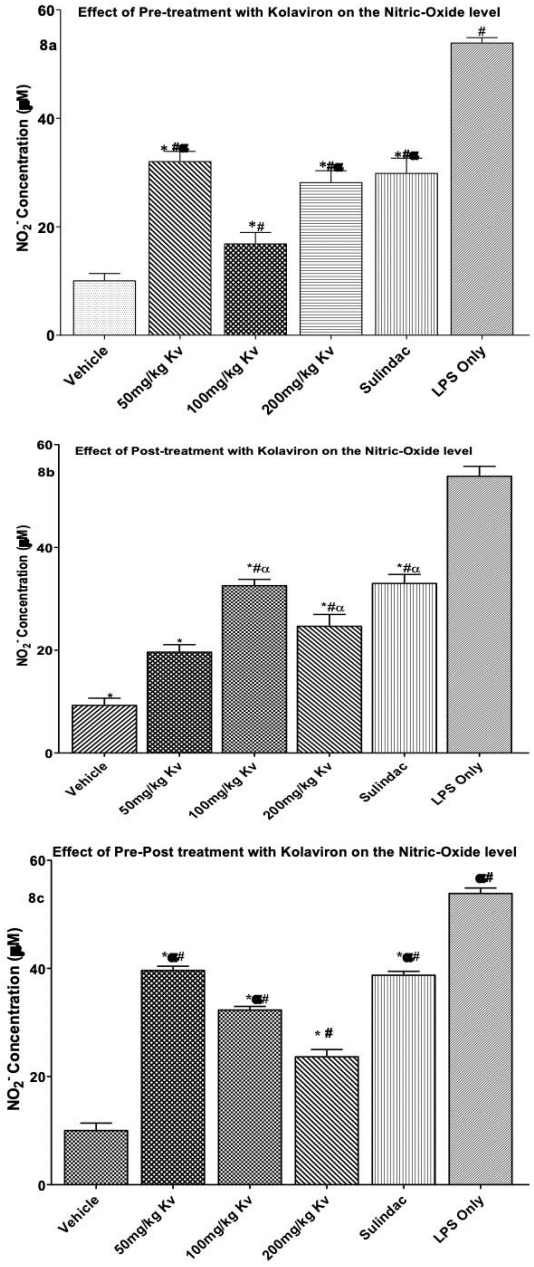

Figure 8:
Effects of Kol-v on single dose of LPS (250µg/kg)-induced cognitive decline on NO_2 analysis the level of the nitric oxide was high in the case of negative control animals (LPS group) which were significantly reduced by the administration of post-treatment with varying concentration of Kolaviron (50mg/kg, 100mg/kg and 200mg/kg) with more significant at Kol-v 100mg/kg for Pre-treatment, 50mg/kg for Post-treatment and 200mg/kg for Pre-Post treatment. *****p versus positive control group (the LPS-stimulated group) **#**p versus negative group (the Vehicle Corn oil only), **α**p versus 50mg/kg, 100mg/kg and 200mg/kg Kol-v respectively. Values are mean ± SEM P< 0.0001; N = 6.

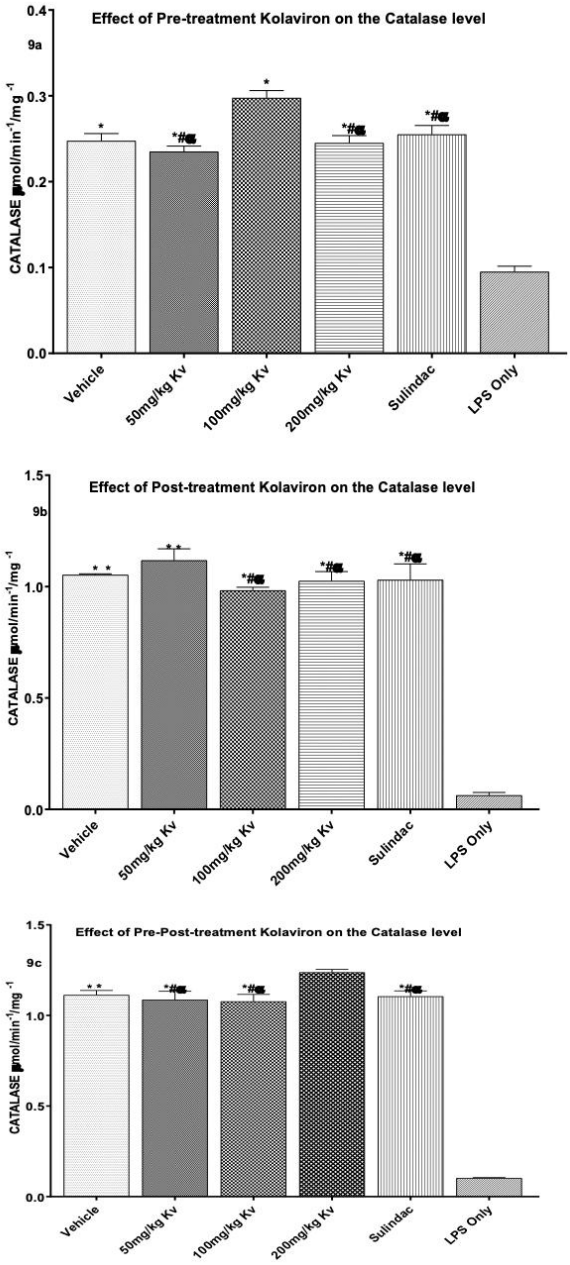

Figure 9:
Effects of Kol-v on single dose of LPS (250µg/kg)-induced cognitive decline on SOD analysis, the Catalase level was reduced in the LPS group which were significantly increased by the post-treatment with Kolaviron (50mg/kg, 100mg/kg and 200mg/kg) with more significant at Kol-v 100mg/kg for Pre-treatment, 50mg/kg for Post-treatment and 200mg/kg for Pre-Post treatment. . *****p versus positive control group (the LPS-stimulated group) **#**p versus negative group (the Vehicle Corn oil only), **α**p versus 50mg/kg, 100mg/kg and 200mg/kg Kol-v respectively. Values are mean ± SEM P < 0.0001; N = 6

Learning and memory tasks was assessed in the test trial (acquisition training), while the long-term memory was assessed in probe trial (non-visible platform trial) In these experiments, Kol-v improved cognitive and learning processes, as shown by a decrease in escape latency in the Morris water maze during training and increase in time spent in quadrant during retrieval. The effect of Kol-v was seen to be effective at 100mg/kg for pre-treatment, 50mg/kg for post-treatment and 200mg/kg for pre- and post-treatment. As reported that probe trials may be more accurately described as extinction procedures (Takeda *et al*., 2004; Lattal *et al*., 2003).

There was a marked drop in temperature four hours after LPS injection, this strong link aligns the fact that hypothermic animal have higher neurological deficits and cognitive decline. However the hypothermia observed in the present study may be considered as an adaptive thermoregulatory or a survival response to a systemic inflammation which is correlated with sepsis or stroke. These suggest that body temperature is a prognostic for cognitive decline such as Alzheimer's and most neurological disease. Furthermore, LPS produced a marked reduction in body weight, noticeable at the first time point of 4 hours. The reduction in body weight is a sign of

inflammatory disease because LPS acts as the hypothalamic center for energy homeostasis (Cia *et al*., 2009). This effect is in agreement with previous reports, showing that relatively high doses of LPS induced weight loss (Kwang *et al*., 2013; Aubert*et al*., 2005; Lugarini*et al*., 2002; Plata-Salaman *et al*., 1993).

The brain is particularly susceptible to oxidative stress, which is explained by its relatively low levels of antioxidants, high concentration of polyunsaturated fatty acids, along with an increased oxygen demand of the brain. Oxidative stress is considered as a baleful condition for normal brain functioning since the brain uses reactive species, which differ chemically for signal transmission. Oxidative stress has been thought to be one of the major processes in development of a wide range of diseases including Alzheimer's disease (Yirmiya *et al*., 2001; Christen *et al*., 2000) and neuro-degeneration in motor neuron diseases (Nunomura *et al*., 2006). Several antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase protect DNA from oxidative stress. Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Khan*et al*., 2010; Di Matteo*et al*., 2003). The present results showed changes in biomarkers of oxidative damage, resulting in a higher oxidative imbalance when induced with LPS.

Higher levels of MDA was seen in the LPS group compared to Kol-v treated groups, which is a major product of the reactive species attack on polyunsaturated fatty acids, and is widely used as a biomarker of lipid peroxidation. It is one of the distinctive features of neuro-degeneration because the brain has high lipid content, after the adipose tissue, thus, elevated serum levels of lipid peroxidation products have been often reported in brain disorders. MDA is a reliable marker for determining oxidative stress in clinical situations and due to MDA's high reactivity and toxicity; treatment with varying Kol-v, reduced the level of lipid peroxides indicating the effective anti-oxidative properties of Kol-v in the moderation of tissue damage in the LPS-stimulated rats at varying concentration. We observed that animals treated with varying concentration of Kol-v especially 100mg/kg for Pre-treatment, 50mg/kg for Post-treatment and 200mg/kg for Pre-Post treatment decreased the lipid peroxidation leading to an increase in GSH, since GSH plays a vital in the role of cellular oxidant defense and is indispensable to demobilize lipid peroxidation (Fulvio *et al*., 2020).

The antioxidant glutathione (GSH) is essential for the cellular detoxification of reactive oxygen species in brain cells. An impaired GSH system in the brain has been intertwine with the oxidative stress occurring in neurological diseases. Recent data demonstrate that besides intracellular functions GSH has also important extracellular functions in brain. The tripeptide glutathione and its related enzymes partake in conserving oxidant homeostasis in aerobic cells. Various biomolecules with redox dependent activity are postulated in the neuronal plasticity events that have a role in learning and memory functions. The up-keep of normal glutathione level is essential for acquisition, but not consolidation, of spatial memory. Glutathione unavailability leads to failures in hippocampal synaptic plasticity mechanisms, which are conceivably related to spatial memory deficit. After LPS administration, there was a decrease in glutathione level that was seen in the LPS-untreated group and Kol-v treated group, this was reflected during the acquisition and probe trial. Cognitive decline have been considered to be an indicator of increased oxidative stress. Kolaviron was more significant at 100mg/kg for Pre-treatment, 50mg/kg for Post-treatment and 200mg/kg for Pre-Post treatment in elevating the GSH level in the experimental rats. It was observed that there was increase in expression of this antioxidant enzyme in cognitive declined rats, which implies that Kol-v has a potential oxidative property for defense activated with the capability to generate detoxification through enhanced scavenging of oxy-radicals and GSH plays an important role in cognition and memory enhancement.

Catalase (CAT) is a heme protein which catalyzes the reduction of hydrogen peroxides and protects the tissues from hydroxyl radicals. Catalase is one of the essential antioxidant enzymes that play a vital role by breaking down hydrogen peroxide and maintaining the cellular redox homeostasis. Catalase has a prime role in regulating the cellular level of hydrogen peroxide and its hydrogen peroxide catabolism protects the cells from oxidative assault (Habib *et al*., 2010). Catalase is a key enzyme which uses hydrogen peroxide, a non-radical ROS, as its substrate. This enzyme is responsible for negating by decomposing hydrogen peroxide, to maintain an optimum level of the molecule in the cell which is also essential for cellular signaling processes. A deficiency in CAT has been associated with many diseases such as diabetes mellitus, cardiovascular diseases, hypertension, anemia, Alzheimer's disease, bipolar disorder, and schizophrenia (Al-Abrash *et al*., 2000). Catalase enzyme was implicated in mutagenesis and inflammation conditions as well as during the suppression which are all known to be associated with oxidative stress conditions. There was decrease in the CAT level after LPS injection which we observed during the learning and memory tasks affecting cognition as CAT is known to play an important role in learning and tolerance to oxidative stress as an adaptive response. There is a closed link between catalase metabolism and oxidant homeostasis, which is expressed during the catalytic decomposition of hydrogen peroxide H_20_2 to water and oxygen which is formed as a by-product of numerous oxidases and SOD reactions.

Superoxide Dismutase (SOD) is the only antioxidant enzyme that salvages the superoxide anion by converting this free radical to oxygen and hydrogen peroxide, thus preventing peroxynitrite production and further damage. Free radicals are strongly associated with many pathological processes in the body. Due to this scavenging ability, SOD has acquired significant attention for therapeutic use. Superoxide dismutase is extensively researched, and used in anti-inflammatory, antitumor, radiation protection, and antisenility applications (Luisa *et al*., 2002). The physiological importance of SOD is illustrated by the severe pathologies evident in mice genetically engineered to lack these enzymes (Lob *et al*., 2002). Mice lacking SOD die several days after birth, amid massive oxidative stress (Seguí *et al*., 2004). Treatment with Kol-v with more significant at 100mg/kg for Pre-treatment, 50mg/kg for Post-treatment and 200mg/kg for Pre-Post treatment

significantly reduced the metabolism of oxidative stress by increasing SOD and CAT level in the LPS induced rats treated with Kol-v.

Nitric oxide signaling in the brain has been reported to regulate different processes (long-term potentiation and depression, LTP and LTD) controlling rhythmic activity, involvement in learning and memory mechanisms through mediation of specific forms of LTP in the cerebellum (Jacoby *et al*., 2001), hippocampus (Garthwaite *et al*., 2008) and neo-cortex, and LTD in the cerebellum. These findings provide strong support to the concept that NO plays a vital role in both learning process and memory of the learnt task (Choopani *et al*., 2008). Since NO is known to relax blood vessels and to increase blood supply to the brain, we accessed the action of NO role in inducing neuronal activity. In this study, a decrease in NO synthesis following an inhibition of NOS activity after LPS injection resulted in vasoconstriction and a decrease in perfusion into the brain. The sympathetic nervous system initially responds to hypothermia by triggering peripheral vasoconstriction, hypertension, tachycardia, and increased cardiac output, justifying the reduction in body temperature after LPS injection when we accessed it. This effect of NOS inhibitors can be proposed for an impairment of learning and memory processes in animals. We established evidence for the involvement of NO in learning and memory processes, the experimental findings demonstrated synthesis of NO and the neuronal action of NO during the period when the animals were trained to learn, and then to remember during the probe trial in the Morris Water Maze. The cognitive effects of LPS increased NO concentration while Kol-v was able to ameliorate its effects by decreasing NO concentration. This is because an increased synthesis of NO has been found to produce neurotoxicity due to accumulation of its toxic metabolite, peroxynitrite which can be a further source of hydroxyl radicals (Massaad 2011). These findings provide strong support to the concept that NO plays a vital role in both learning process and memory of the learnt task; which indicates the anti-inflammatory properties of Kol-v as earlier suggested (Olaleye *et al*., 2010).

In conclusion, we can establish a relationship between the progress of cognitive decline and increased oxidative stress with lipopolysaccharide. Kolaviron showed significant anti-inflammatory potentials through its protection against cognitive decline and oxidative properties, induced by lipopolysaccharide in the laboratory Wistar rat. Also, Kolaviron showed protection against oxidative stress in the laboratory Wistar rat.

REFERENCES

Abarikwu, S. O. (2014). Kolaviron, a natural flavonoid from the seeds of Garcinia kola, reduces LPS-induced inflammation in macrophages by combined inhibition of IL-6 secretion, and inflammatory transcription factors, ERK1/2, NF-?B, p38, Akt, p-c-JUN and JNK. Biochem Biophys Acta. 1840: 2373 - 2381.

Abarikwu, S. O., Farombi, E. O. and Pant, A. B. (2011). Biflavanone-kolaviron protects human dopaminergic SH-SY5Y cells against atrazine induced toxic insult. ToxicolIn Vitro. 25: 848 - 858.

Adaramoye, O. A. (2010). Protective Effect of Kolaviron, a Biflavonoid from Garcinia kola Seeds, in Brain of Wistar Albino Rats Exposed to Gamma-Radiation. Biological & Pharmaceutical Bulletin, 33: 260 - 266.

Arai, K., Matsuki, N., Ikegaya, Y. and Nishiyama, N. (2001). Deterioration of spatial learning performances in lipopolysaccharide treated mice, Jpn J Pharmacol. 87: 195 - 201.

Aubert, A. and Dantzer, R. (2005). The taste of sickness: lipopolysaccharide-induced finickiness in rats. Physiol Behav. 84: 437–444.

Barrientos, R. M., Frank, M. G., Hein, A. M. *et al.,* (2009). "Time course of hippocampal IL-1$\beta$and memory consolidation impairments in aging rats following peripheral infection," Brain, Behavior, and Immunity. 23(1): 46 – 54.

Beuge, J. A. and Aust, S. D. (1978). Microsomal Lipid peroxidation. Mehod Enzymol. 30: 302-310.

Bluthe, R. M., Castanon, N., Pousset, F., *et al*. (1999). Central injection of IL-10 antagonizes the behavioral effects of lipopolysaccharide in rats. Psychoneuroendocrinology; 24: 301–311.

Choopani, S., Moosavi, M., and Naghdi, N. (2008). Involvement of nitric oxide in insulin-induced memory impairment. Peptides. 29: 898–903. [PubMed] [Google Scholar]

Christen, Y. (2000). Oxidative stress and Alzheimer disease, Am J Clin Nutr.71(2): 621S–629S.

Cia, G. and Freeman, G. J. (2009) The CD160, BTLA, LIGHT/HVEM pathway: a bidirectional switch regulating T-cells activation. Immunol Rev. 229: 244-58.

Clairborne, A. (1995). Catalase Activity. In handbook of Methods for Oxygen Radical Research. A .R Greewald Editors. CRC Boca Raton, Florida, USA 237-242.

Das, S., Vasisht, S., Snehlata, R., Das, N. and Srivastava, L. M. (2000). Correlation between total antioxidant status and lipid peroxidation in hypercholesterolemia, Curr Sci. 78: 486-487.

Di Matteo, V. and Esposito, E. (2003). Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, Curr Drug Targets CNS NeurolDisord 2(2) 95–107.

Farokhnia, M., ShafieeSabet, M., Iranpour, N., Gougol, A., Yekehtaz, H., Alimardani, R., Farsad, F., Kamalipour, M., Akhondzadeh, S. (2014). Comparing the efficacy and safety of Crocus sativus L. with memantine in patients with moderate to severe Alzheimer's disease: a double-blind randomized clinical trial. Hum Psychopharmacol. 29: 351-359.

Farombi, E. O., Shrotriya, S. and Surh, Y. J. (2009). Kol aviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-kappa B and AP-1. Life Sci. 84: 149-155.

Fulvio, U. and Matilde, M. (2020). Lipid peroxidation and ferroptosis: The role of GSH and GPx4, Free Radical Biology and Medicine, Volume 152, , Pages 175-185, ISSN 0891-5849,https://doi.org/10.1016/j.freeradbiomed.2020.02.027.

Garthwaite, J.,(2008)**.** Concepts of neural nitric oxide-mediated transmission.*Eur J Neurosci*.; 27 2783-802.

Griess, P. (1879) Bemerkungen zu der abhandlung der H.H. Weselsky und Benedikt Uebereinige azoverbindungen." *Chem. Ber.* **12**, 426–8.

Hiner, A., Raven, E., Thorneley, R., García-Cánovas, F. and Rodríguez-López, J. (2002). "Mechanisms of compound I formation in heme peroxidases". J Inorg Biochem. 91 (1): 27–34.

Hrupka, B. J. and Langhans, W.(2001). A role for serotonin in lipopolysaccharide-induced anorexia in rats.PharmacolBiochemBehav.; 68 355–362.

Igado, O. O., Olopade, J. O., Adesida, A., Aina, O. O., Farombi, E. O. (2012). Morphological and biochemical investigation into the possible neuroprotective effects of kolaviron (*Garcinia kola* bioflavonoid) on the brains of rats exposed to vanadium. Drug Chem Toxicol. 35: 371-380.

Ijomone, O. M., Nwoha, P. U., Olaibi, O. K., Obi, A. U. and Alese, M. O. (2012). Neuroprotective Effects of Kolaviron, a Biflavonoid Complex of Garcinia kola, on Rats Hippocampus

against Methamphetamine-Induced Neurotoxicity. Macedonian Journal of Medical Sciences, 5: 10-16.

Iwu, M. M. (1985). Antihepatoxic constituents of Garcinia kola seeds. Experientia 41: 699-700.

Jacoby, S., Sims, R. E. and Hartell, N. A. (2001). Nitric oxide is required for the induction and heterosynaptic spread of long-term potentiation in rat cerebellar slices. J Physiol. 535: 825-839.

Jollow, D. J., Mitchell, J. R., Zampaglione, N. and Gillette, J. R. (1974). Bromobenzene induced liver necrosis protective role of glutathione and evidence for 3,4, Bromobenzene oxide as the hepatoxic metabolite. Pharmacology 135: 151-169.

Lattal, K. M., Mullen, M. T. and Abel, T. (2003). Extinction, renewal, and spontaneous recovery of a spatial preference in the water maze. BehavNeurosci. 117(5): 1017-1028.

Lee, Y, Kim MS, Lee J2017.Neuroprotective strategies to prevent and treat Parkinson's disease based on its pathophysiological mechanism. Arch Pharm Res. Oct; 40(10):1117-1128.

Lee, J., Torosyan, N. and Silverman, D. H. (2017). Examining the impact of grape consumption on brain metabolism and cognitive function in patients with mild decline in cognition: a double-blinded placebo controlled pilot study. Exp. Gerontol. 87: 121-128.

Lob, H. E., Marvar. P. J., Guzik, T.J., Sharma, S., McCann, L. A., Weyand, C, Gordon, F. J. and Harrison, D. G. (2010). "Induction of hypertension and peripheral inflammation by reduction of extracellular superoxide dismutase in the central nervous system". Hypertension. 55 (2): 277–83, 6p following 283. doi:10.1161/HYPERTENSIONAHA.109.142646. PMC 2813894. PMID 20008675.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L and Randall, R.J. (1951). Protein measurement with folin phenol reagent. J. Biol. Chem. 193: 265-275.

Lugarini, F., Hrupka, B. J., Schwartz, G. J., Plata-Salaman, C. R. and Langhans, W. (2002). A role for cyclooxygenase 2 in lipopolysaccharide-induced anorexia in rats. Am J PhysiolRegulIntegr Comp Physiol. 283: R862–R868.

Mao, X. Y., Jin, M. Z., Chen, J. F., Zhou, H. H., Jin, WL. (2018). Live or let die: Neuroprotective and anti-cancer effects of nutraceutical antioxidants. Pharmacol Ther. 183: 137-151.

Misra, H. P., and Fridovish, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol. Chem. 247: 3170-3175

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods, 11: 47-60.

Nunomura, A., Castellan, R., Zhu, X., Moreira, P., Perry G. and Smith, M. (2006). Involvement of oxidative stress in Alzheimer disease, J NeuropatholExp Neurol. 65(7): 631–641.

Nwoha, P. U., Ojo, B., Ajayi, S. A., Ofusori, D. A., Oluwayinka, O. P., Odukoya, S. A. and Falana, B. A. (2007). Garcinia kola Diet Provides Slight Protection to Mice Hippocampal Neurons against Neuro-Toxins. J. Environ Neurosci. Biomed. 1: 125-136.

Olajide, O. J., Enaibe, B. U., Bankole, O. O., Akinola, O. B., Laoye, B. J., Ogundele OM (2015) Kolaviron was protective against sodium azide (NaN3) induced oxidative stress in the prefrontal cortex. Metab Brain Dis. 31(1): 25-35.

Olaleye, S. B., Onasanwo, S. A., Ige, A. O., Wu, K. K. and Cho, C. H. (2010) Anti-inflammatory activities of a kolaviron-inhibition of nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha production in activated macrophage-like cell line. Afr J Med Med Sci. Suppl 39: 41-46.

Onasanwo, S. A., Velagapudi, R., El-Bakoush, A. and Olajide, O. A. (2016). Inhibition of neuroinflammation in BV2 microglia by the biflavonoidkolaviron is dependent on the Nrf2/ARE antioxidant protective mechanism. Mol Cell Biochem. 414(1-2): 23-36.

Palumbo, M. L., Fosser, N. S., Rios, H., Zorrilla Zubilette MA, Guelman, L. R., *et al*. (2007). Loss of hippocampal neuronal nitric oxide synthase contributes to the stress-related deficit in learning and memory..,J Neurochem. 102: 261–74. [PubMed] [Google Scholar]

Plata-Salaman, C. R. and Borkoski, J. P. (1993). Centrally administered bacterial lipopolysaccharide depresses feeding in rats. PharmacolBiochem Behav. 46: 787–791.

Rao, A. and Balachandran, B. (2002). Role of oxidative stress and antioxidants in neurodegenerative diseases, Nutr Neurosci. 5 (5): 291–309.

Rosi, S., Vazdarjanova, A., Ramirez-Amaya, V., Workey, P. F., Barnes, C. A. and Wenk, G. L. (2006). Memantine protects against LPS-induced neuroinflammation, restores behaviourally-induced gene expression and spatial learning in the rat. Neurosci. 142: 1303-1315.

Seguí, J., Gironella, M., Sans, M., Granell, S., Gil, F., Gimeno, M., Coronel, P., Piqué, J. M. and Panés, J. (2004). "Superoxide dismutase ameliorates TNBS-induced colitis by reducing oxidative stress, adhesion molecule expression, and leukocyte recruitment into the inflamed intestine". Journal of Leukocyte Biology. 76 (3): 537–44. doi:10.1189/jlb.0304196. PMID 15197232. S2CID 15028921.

Sorce, S., Stocker, R., Seredenina, T,, Holmdahl, R., Aguzzi, A., Chio, A., Depaulis, A., Heitz, F., Olofsson, P., Olsson, T., Duveau, V., Sanoudou, D., Skosgater, S., Vlahou, A., Wasquel, D., Krause, K. H., Jaquet, V. (2017). NADPH oxidases as drug targets and biomarkers in neurodegenerative diseases: What is the evidence? Free RadicBiol Med. 112: 387-396.

Takeda, K. and Akira, S. (2004). TLR signaling pathways. Seminars in Immun. 16: 3-9.

Ullah, F., Liang, A., Rangel, A., Gyengesi, E., Niedermayer, G., Münch, G. (2017). High bioavailability curcumin: an anti-inflammatory and neurosupportive bioactive nutrient for neurodegenerative diseases characterized by chronic neuroinflammation. Arch Toxicol. 91: 1623-1634.

Van Eldik, L. J., Carrillo, M. C., Cole, P. E., Feuerbach, D., Greenberg, B. D., Hendrix, J. A., Kennedy, M., Kozauer, N., Margolin, R. A., Molinuevo, J. L., *et al*. (2016). The roles of inflammation and immune mechanisms in Alzheimer's disease.Alzheimer's Dement. Transl. Res. Clin. Interv. 2: 99–109.

Yirmiya, R., Pollak, Y., Barak, O., Avitsur, R., Ovadia, H., Bette, M., Weihe, E. and Weidenfeld, J. (2001). Effects of antidepressant drugs on the behavioral and physiological responses to Lipopolysaccharide (LPS) in rodents, Neuro-psychopharmacology. 24: 531–544.